Affymetrix® Package Insert

GeneAtlas™ Hybridization, Wash, and Stain Kit for WT Array Strips

60 Reactions

Intended Use

This package insert provides information for the GeneAtlas™ Hybridization, Wash, and Stain Kit for WT Array Strips (P/N 901667). The kit is composed of two boxes:

- P/N 901668: GeneAtlas™ Hybridization Module for WT Array Strips – sufficient for 60 reactions
- P/N 901625: GeneAtlas™ Wash Buffers A and B Module – sufficient for 60 array strips.

The GeneAtlas Hybridization, Wash, and Stain Kit for WT Array Strips includes all the necessary reagents for the hybridization, wash, and stain steps of the Ambion WT Expression Kit and GeneChip WT Terminal Labeling and Controls Kit using Affymetrix® Whole Transcript (WT) Array Strips.

Module Components

Component	Volume	Storage
GeneAtlas™ Hybridization Module for WT Array Strips (P/N 901668)		
5X WT Hyb Add 1 (P/N 901618)	3 x 925 µL	2°C to 8°C
15X WT Hyb Add 4 (P/N 901619)	3 x 355 µL	2°C to 8°C
2.5X WT Hyb Add 6 (P/N 901620)	3 x 1,525 µL	2°C to 8°C
Stain Cocktail 1 (P/N 901541)	52.5 mL	2°C to 8°C
Stain Cocktail 2 (P/N 901542)	26.3 mL	2°C to 8°C
Array Holding Buffer (P/N 901543)	15 mL	2°C to 8°C
GeneAtlas™ Wash Buffers A & B Module (P/N 901625)		
Wash Buffer A	519 mL	2°C to 8°C
Wash Buffer B	64 mL	2°C to 8°C

Note: The "WT Hyb Add" reagent names were created to match the order in which reagents are added. For example, WT Hyb Add 4 is the fourth component added during preparation of the Hybridization Mix. WT Hyb Add 2, 3 and 5 are not used and are not part of the Hybridization Modules.

Shipping Information

The components of the GeneAtlas Hybridization, Wash, and Stain Kit are shipped at different temperatures. The GeneAtlas™ Hybridization Module for WT Array Strips (P/N 901668) will be sent at 2°C to 8°C. The GeneAtlas™ Wash Buffers A & B Module (P/N 901625) will be sent at ambient temperature.

Safety Information

Warning! P/N 901668 contains Tetramethylammonium chloride (TMACl) and formamide, both of which are considered highly toxic. P/N 901668 contains Sodium Azide. May react with lead and copper plumbing to form highly explosive metal azides. A Material Safety Data Sheet(s) (MSDS) is available at www.affymetrix.com. If the product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Functional Testing

Each lot of reagents is rigorously tested for performance.

Ordering Information

P/N	Product Name	Description/Size
901667	GeneAtlas™ Hybridization, Wash, and Stain Kit for WT Array Strips	60 Rxn
Related Products		
4411973	Ambion® WT Expression Kit	10 Rxns
900670	GeneChip® WT Terminal Labeling Kit	10 Rxns

Affymetrix products can be purchased directly from Affymetrix in the United States, many European countries, and many Asian countries. For all other territories, please view a list of our distribution partners, which can be located at: www.affymetrix.com/site/contact/index.affx.

Contact Information

Affymetrix, Inc.
3420 Central Expressway
Santa Clara, CA 95051 USA
E-mail: support@affymetrix.com
Tel: 1-888-362-2447 (1-888-DNA-CHIP)
Fax: 1-408-731-5441

Affymetrix UK Ltd
Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH
United Kingdom
E-mail: supporteurope@affymetrix.com
UK and Others Tel: +44 (0) 1628 552550
France Tel: 0800919505
Germany Tel: 01803001334
Fax: +44 (0) 1628 552585

Affymetrix Japan, K. K.
ORIX Hamamatsucho Bldg, 7F
1-24-8 Hamamatsucho, Minato-ku
Tokyo 105-0013 Japan
E-mail: supportjapan@affymetrix.com
Tel: +81-3-6430-4020
Fax: +81-3-6430-4021

Please visit our web site for international distributor contact information
www.affymetrix.com

Limited License

Subject to the Affymetrix terms and conditions that govern your use of Affymetrix products, Affymetrix grants you a non-exclusive, non-transferable, non-sublicensable license to use this Affymetrix product only in accordance with the manual and written instructions provided by Affymetrix. You understand and agree that except as expressly set forth in the Affymetrix terms and conditions, that no right or license to any patent or other intellectual property owned or licensable by Affymetrix is conveyed or implied by this Affymetrix product. In particular, no right or license is conveyed or implied to use this Affymetrix product in combination with a product not provided, licensed or specifically recommended by Affymetrix for such use.

Trademarks

Affymetrix®, Axiom™, Command Console®, DMET™, GeneAtlas™, GeneChip®, GeneChip-compatible™, GeneTitan®, Genotyping Console™, NetAffx®, and Powered by Affymetrix™ are trademarks or registered trademarks of Affymetrix Inc. All other trademarks are the property of their respective owners.

Copyright

© 2010 Affymetrix, Inc. All rights reserved.

Hybridization Cocktail Preparation Instructions for Processing Gene 1.1 ST Array Strips using the GeneAtlas™ Instrument and the WT Assay

The Hybridization Mix described in this Quick Reference Guide was specifically formulated for use with the Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit.

Note: The "WT Hyb Add" reagent names were created to match the order in which reagents are added. For example, WT Hyb Add 4 is the fourth component added during preparation of the Hybridization Mix. WT Hyb Add 2, 3 and 5 are not used and are not part of the Hybridization Modules.

1. Remove the vials labeled **5X WT Hyb Add 1**, **15X WT Hyb Add 4** and **2.5X WT Hyb Add 6** from the GeneAtlas Hybridization Module for WT Array Strips, P/N 901668.
   - **A.** Warm reagents to room temperature on the bench.
   - **B.** Vortex the **5X WT Hyb Add 1**, **15X WT Hyb Add 4** and **2.5X WT Hyb Add 6** to mix. Centrifuge briefly (~5 sec) to collect liquid at the bottom of the tube.
2. Remove the GeneChip® Hybridization Control Kit from –20°C freezer and thaw at room temperature.
   - **A.** Vortex and centrifuge briefly (~5 sec) to collect liquid at the bottom of the tube.
   - **B.** Keep on ice.
3. **WT Hybridization Master Mix**: A new hybridization mix has been formulated for the Ambion WT Expression Kit and the GeneChip® WT Terminal Labeling and Controls Kit.
   - **A.** Prepare the WT Hybridization Master Mix in the order as shown in Table 1. The **5X WT Hyb Add 1** solution is very viscous, pipet slowly to ensure addition of the correct volume. Mix well.

Table 1

Order to Add Reagents	Component	Volume per Array	4-Array Strip*	Final Concentration
1	5X WT Hyb Add 1	30 µL	132 µL	1X
2	Control Oligonucleotide B2 (3 nM)	1.5 µL	6.6 µL	30 pM
3	20X Eukaryotic Hybridization Controls (*bioB, bioC, bioD, cre*)	7.5 µL	33 µL	1.5, 5, 25 and 100 pM, respectively
4	15X WT Hyb Add 4	10 µL	44 µL	1X
Total Volume		**49 µL**	**215.6 µL**	

**Includes ~ 10% overage to cover pipetting error.*

   - **B.** Aliquot 49 µL of the master mix prepared in Table 1 to each tube or well. Add the fragmented and labeled single-stranded DNA target generated from the *GeneAtlas™ WT Expression Kit User Manual* (P/N 702935) as shown in Table 2.

Table 2

Order to Add Reagents	Component	Volume per Array	Final Concentration
5	Fragmented and Labeled DNA	41 µL	~25 ng/µL
Total Volume		**90 µL**	

   - **C.** Add the **2.5X WT Hyb Add 6** from the GeneAtlas Hybridization Module for WT Array Strips as shown in Table 3.

Table 3

Order to Add Reagents	Component	Volume per Array	Final Concentration
6	2.5X WT Hyb Add 6	60 µL	1X
Total Volume		**150 µL**	

   - **D.** If you are using a plate: seal, vortex and centrifuge briefly (~5 sec) to collect liquid at the bottom of the well. If you are using 1.5 mL tubes: vortex and centrifuge briefly (~5 sec) to collect liquid at the bottom of the tube.
4. Denature the hybridization cocktail with target at 99°C (1.5 mL tubes) or 95°C (thermocycler plates) for 5 minutes, followed by 45°C for 5 minutes.
5. After denaturation, spin hybridization cocktail with target in a centrifuge to remove any insoluble material from the hybridization mixture. If you are using 1.5 mL tubes, use an Eppendorf 5417C centrifuge (or equivalent). If you are using thermocycler plates, use an Eppendorf 5804R centrifuge (or equivalent). Spin tubes or plates for 1 minute at 5000 RPM at room temperature.
6. Place 120 µL of the centrifuged supernatant hybridization cocktail with target into the appropriate well of the hybridization tray.
7. Refer to the *GeneAtlas™ System User's Guide* (P/N 08-0306) for details on the GeneAtlas hybridization setup.

For Research Use Only. Not for use in Diagnostic Procedures.